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Plasma volume expansion in rats: effects on thermoregulation and exercise

R. P. FRANCESCONI, M. BOSSELAERS, C. MATTHEW, AND R. W. HUBBARD
US Army Research Institute of Environmental Medicine, Natick, Massachusetts 01760-5007

FRANCESCONI, R. P., M. BOSSELAERS, C. MATTHEW, AND R. W. HUBBARD. *Plasma volume expansion in rats: effects on thermoregulation and exercise.* J. Appl. Physiol 66(4): 1749–1755, 1989.—Administration of polyethylene glycol (PEG, intraperitoneal, 3 ml, 30% solution) to adult male rats (300 g) resulted in an ~20% increment in plasma volume (PV) 24 h after PEG injection. When these animals were exercised (9.14 m/min, level treadmill) in a warm (30°C, 30–40% relative humidity) environment, their mean endurance was increased from 67.9 (saline-treated controls, CONT) to 93.6 min ($P < 0.01$). Total water loss was increased from 12.2 (CONT) to 17.2 g (PEG, $P < 0.01$). Atropine administration (ATR, 200 μg/kg, tail vein) significantly ($P < 0.05$) reduced both the endurance and the salivary water loss of CONT and PEG-treated rats, whereas it increased the heating rate ($P < 0.01$) of both groups. PEG treatment reduced ($P < 0.01$) the hematocrit and circulating protein levels both before and subsequent to exercise in the warm environment. Clinical chemical indexes of heat/exercise injury were generally unaffected by pharmacological intervention, whereas clinical chemical responses to exercise were related to the endurance time of each group. We concluded that expansion of PV by PEG provided significant beneficial effects on performance and thermoregulation during exercise in a warm environment.

polyethylene glycol; atropine; salivation; physical performance; indexes of heat injury

FOR A NUMBER OF YEARS we have been interested in the identification and investigation of pharmacological, physiological, or training interventions that may be effective in reducing the physiological cost of work in the heat, increasing heat dissipation during work in the heat, or increasing heat/exercise endurance. Similarly, we have undertaken research designed to identify and quantitate the debilitating effects of factors that predispose animals or humans to heat injury. To this end we have reported the decremental effects on exercise in the heat of a low-potassium diet (16), alcohol consumption (10), preinduced hyperthermia (11), phenothiazine administration (9), and acute pyridostigmine bromide administration (5). Alternatively, we have documented the beneficial effects on exercise in the heat of preinduced hypothermia elicited by acute administration of tryptophan (7) or a glucose analogue (8) followed by acute cold exposure. In continuing this line of research, the current investigation was designed to evaluate the effects of marked hyperhydration on endurance and thermoregulation during exercise in a warm environment.

In an early paper, Stricker (26) used the subcutaneous administration of hyperosmotic polyethylene glycol (PEG, 10 or 30% solution) and inferior vena caval ligation to "elicit more drinking with fluid retention than any other experimental procedure known." However, he further reported that 0.15 M NaCl was consumed in greater quantities than water over the ensuing 24-h period. Initially, the extravascular administration of PEG to rats is followed by a period in which water is removed from the intravascular space under the strong osmotic influence of the exogenously administered PEG (27). This period persists for at least 8 h during which plasma volume deficits persisted as evidenced in hematocrit levels from 55 to 60%, urine outputs were low, and fluid consumption was high (25). However, during a 24-h period after the subcutaneous administration of 5 ml of 30% PEG, Stricker (27) has reported that total fluid consumption may be as high as 55 ml, and urinary output was ~15 ml, whereas in a group of control animals fluid consumption was ~25 ml, and urine output was ~22 ml. Furthermore, Stricker and MacArthur (29) have reported that when PEG was administered intraperitoneally, PEG is found in the plasma between 12 and 18 h, and plasma volume first equilibrates and then begins to expand under the influence of the increased drinking with fluid retention, the osmotic effect of the PEG within the intravascular space, and the greatly increased concentrations of plasma hormones subserving water reabsorption and electrolyte retention (28). Physiological differences in the effects of intraperitoneally and subcutaneously administered PEG may be due to the presence of major lymphatics underlying the diaphragm and draining the peritoneal cavity that transport the PEG to the vascular system.

After the uptake of 1–2% PEG into the intravascular volume from the intraperitoneal injectate, Stricker and MacArthur (29) concluded that this PEG would provide a strong osmotic influence in promoting the transfer of interstitial fluid to the intravascular space and reabsorption of water from the proximal tubules of the kidney, leading to oliguria. Thus, even without greatly stimulated water consumption, explanations can be provided for significantly elevated plasma volume even while urine volume is markedly reduced. The time course of these alterations is of obvious importance, and our preliminary work indicated that 24 h provided adequate time for sufficient but not excessive hemodilution.

Thus Stricker and MacArthur (29) reported that 24 h

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after intraperitoneal administration of PEG, mean hematocrit levels were reduced from 45 ± 0.8% to ~41%, whereas in wild rabbits receiving 4 g/kg body wt of 30% PEG, hematocrits were reduced from 43 to 38% after 48 h and to 32% after 72 h (1). Thus, although not extensively investigated, there appear clear indications that after the acute hypovolemia of PEG administration, there ensues an interval (24–72 h) wherein expansion of plasma volume elicits an apparent intravascular hyperhydrated state. The current study was designed to assess the effects of such plasma volume expansion on physical performance and thermoregulation in a warm environment.

Additionally, rats secrete copious amounts of saliva for evaporative heat loss during exposure to a warm environment (12, 13). However, during exercise this excessive water loss is of questionable physiological benefit due to the inability of the exercising rats to spread this saliva behaviorally for evaporation and consequent heat dissipation (5, 22). We have reported (17, 21) that, in rats, atropine, a potent and widely used anticholinergic, is effective in inhibiting saliva secretion and evaporative water loss when the animals were passively exposed to a hot environment. Although atropine also elicits elevations in heart rate, reductions in gastrointestinal motility, urinary retention, and pupil mydriasis, we have reported (17) the extreme sensitivity of salivary secretion to low doses of atropine. Thus we wished to evaluate the effects of atropine on thermoregulation in an appropriate animal model of human heat/exercise illness (15, 18), especially when plasma volume was markedly increased.

METHODS

Adult male Sprague-Dawley rats (Charles River Breeding Laboratories, Wilmington, MA) were obtained at ~250–275 g and maintained at our facility for 5–7 days or until experimental weight of ~300 g was attained. The animals were housed singly in wire-bottomed cages, and food (Agway 3000) and water were available ad lib. Fluorescent lighting (on from 0600–1800 h) was automatically controlled in a windowless room maintained at 21 ± 1°C, and animals were frequently weighed as they approached experimental weight. Because no effects of training or previous heat exposure were of interest, naive rats were used in all experiments; the slow treadmill speed (9.14 m/min) assured that the vast majority of the animals would run under these conditions without prior training.

At ~48 h before an experimental trial, a Silastic catheter was permanently implanted into the external jugular vein while the animal was anesthetized (pentobarbital sodium, 40 mg/kg body wt) using aseptic techniques. This minor surgical intervention had no effects on the subsequent ability to exercise. At 24 h before an experimental trial, a small sample of blood (200 μl) was removed from the catheter to determine the hematocrit ratio before experimental manipulation. Rats were then randomly divided into four groups as follows: *1*) a control group (CONT, $n = 10$), which received 3.0 ml of sterile physiological saline by intraperitoneal injection 24 h before run and 0.2 ml saline intravenously 30 min before the run; 2) an atropine-treated group (ATR, $n = 9$, 1 animal removed due to high initial temperature), which also received 3.0 ml physiological saline intraperitoneally and 24 h later (i.e., 30 min before an experimental run) was also administered 200 μg/kg ATR sulfate (0.2 ml) by tail vein injection; *3*) a third group received 3.0 ml of 30% PEG solution ($n = 10$) 24 h before an experimental trial and 0.2 ml saline intravenously 30 min before running; and *4*) the final group was injected with 3.0 ml of 30% PEG 24 h before an experimental run and 200 μg/kg ATR (0.2 ml) 30 min before the run (PEG-ATR, $n = 10$).

All experiments were conducted in a large stainless steel chamber set at 30 ± 0.5°C; the treadmill speed was set at 9.14 m/min (0° angle of incline), and a shock-avoidance contingency was employed. Animals ran under these conditions until hyperthermic exhaustion ensued [rectal temperature (T_{re}) = 42°C, animal unable to right itself] or 99 min, whichever occurred first. During the entire exercise interval T_{re} (6 cm) and skin temperature (T_{sk}, tailskin, midlength) were automatically sampled and recorded (HP85 desk-top computer, HP3456A digital volt meter, and HP3495A scanner) at 1-min intervals.

Small blood samples (0.8 ml) were taken in heparinized syringes ~15 min before initiating the run, and hematocrit was immediately determined by microcentrifugation. The plasma fraction from the microhematocrit tube was immediately analyzed for protein content by refractometry. The remainder of the blood sample was centrifuged (4°C, 10,000 *g*), and a fresh plasma sample was stored in ice for osmolality determination (freezing-point depression, μOsmette, Precision Systems). The remainder of the plasma was deep frozen (−20°C) and stored for subsequent analysis of creatine phosphokinase, lactic acid dehydrogenase, lactate, urea nitrogen, and creatinine. All of these assays were performed using a Gilford semiautomated spectrophotometer (Stasar IV) and Gilford diagnostic reagent kits, according to methods outlined in the respective technical bulletins. Sodium and potassium levels were measured by flame photometry (Radiometer, FLM 3). A second blood sample was taken immediately on termination of the treadmill run; this sample was processed, stored, and analyzed exactly as the first.

Statistically significant effects were established by analysis of variance followed by the application of Tukey's test for critical differences of the means (19, 20). Because only nine animals were available in the ATR group, a single calculated value was used for this group (19). The null hypothesis was rejected at $P < 0.05$.

RESULTS

It was initially necessary to estimate the change in plasma volume elicited by the experimental regimen (i.e., 3.0 ml of 30% PEG 24 h before trial). To this end preliminary experiments were performed on blood samples taken immediately before PEG administration and 24 h later. Both blood samples were analyzed for hematocrit and hemoglobin (cyanomethemoglobin method), and percent changes in plasma volume were calculated by the method of Dill and Costill (2). These preliminary

results ($n = 4$) demonstrated that the experimental intervention reduced hematocrit levels from 42.1 ± 0.63 to 36.9 ± 0.51 (SD) and hemoglobin from 14.2 ± 0.3 to 12.65 ± 0.2 (SD) g/100 ml, eliciting a mean calculated percent increment in plasma volume of 21.5 ± 1.4% (range = 18.7–25.1%). We concluded that this dose, route of administration, and time lapse (24 h) provided an optimal range of plasma volume expansion for the current studies.

We also performed preliminary studies on the effects of PEG administration on weight gain, food and water consumption, and urine output. In a separate group of six animals we injected 3 ml of 30% PEG and monitored these variables for the subsequent 24 h. Although mean body weight increased slightly (396 ± 5.0 to 398.5 ± 5.1 g, food consumption was decreased (25.8 ± 3.2 vs. 15.2 ± 1.7 g) significantly after PEG administration. It is important to note, however, that water consumption was actually increased in the 24 h after PEG administration (31 ± 2.8 vs. 33.8 ± 3.3.6 g), whereas urine output was decreased (9.6 ± 0.6 vs. 6.1 ± 1.1 ml).

Figures 1 and 2 demonstrate the effects of the four treatment regimens on thermoregulatory responses (T_{re} and T_{sk}) to exercise in a warm environment. Through the first 30 min of the treadmill exercise, Fig. 1 illustrates no effects of the treatments on T_{re}, whereas at 40 min, mean T_{re} for the ATR group is significantly ($P < 0.01$) elevated compared with the PEG group. This difference ($P < 0.01$) persists and is exaggerated after 50 and 60 min, with the CONT and PEG-ATR groups falling between (without significant difference from) the extremely hyperthermic ATR group and the much cooler PEG groups. T_{sk} (Fig. 2) was elevated by the constant work rate and the warm ambient temperature but was apparently unaffected by any of the pharmacological interventions.

Data depicted in Table 1 confirm what is apparent in Fig. 1—that the rapidly developing hyperthermia of the ATR group resulted in a significantly ($P < 0.05$) reduced endurance capacity compared with that of the saline-treated CONT group. Additionally, the expansion of the intravascular volume of the PEG-treated group led to a significant ($P < 0.01$) increase in physical performance (5 of 10 animals in this group could have continued beyond the 99-min criterion; however, experimental variables in the animals that could have continued were not significantly different from others in the PEG group). Interestingly, the combined PEG-ATR treatment elicited a mean endurance that was significantly ($P < 0.01$) greater than that of the ATR group, significantly ($P < 0.05$) less than that of the PEG-treated animals, and not significantly different from the endurance of the CONT group. The significant decrease in total water loss in the ATR-group ($P < 0.01$ from CONT) and the elevation in the PEG group ($P < 0.01$) obviously had no beneficial effects for the ATR-treated or decremental effects for the PEG-treated rats. Mean weight loss per minute in the PEG-treated rats was not different from controls despite the significantly increased endurance time. As suggested in Fig. 1, increments (ΔT_{re}/min) in T_{re} in the ATR-treated group were significantly higher ($P < 0.01$) than controls, whereas PEG-treated rats manifested significant ($P < 0.05$) decrements in this variable.

Table 2 reports the effects of the treatment regimens and exercise in a warm environment on several indexes of hydrational status. Most importantly, hematocrit levels in both PEG-treated groups were significantly ($P < 0.01$) less than respective control levels before and after exercise. Before exercise, sodium (Na^+) levels were significantly ($P < 0.01$) reduced in the PEG-treated group, but significance was not attained in the PEG-ATR group. Exercise in the warm environment effected significant ($P < 0.05$, minimal) increments in plasma Na^+ in all groups. Although plasma osmolality was increased ($P < 0.01$) in all groups by exercise, the significantly ($P < 0.01$) reduced level in the postexercise sample of the ATR-treated group may be related to the decremented endurance; however, the similar significant ($P < 0.01$)

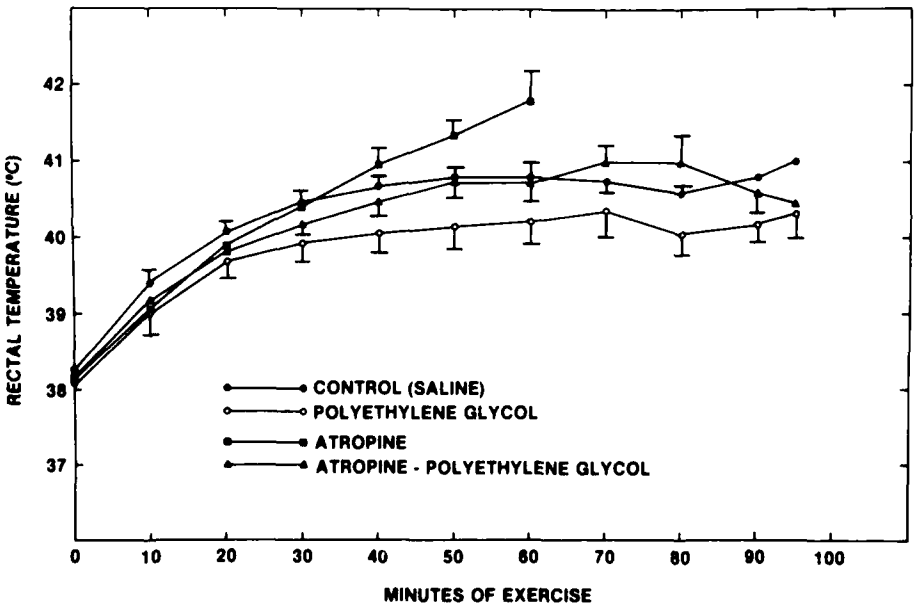

FIG. 1. Effects of polyethylene glycol, atropine, and a polyethylene glycol-atropine combination on rectal temperature responses to mild exercise (9.14 m/min, 0° angle) in a warm environment (30°C). Mean values (SE bars) are depicted for $n = 10$/group, except $n = 9$ for ATR. Exercise was terminated at 99 min or when animals could not continue to maintain the pace.

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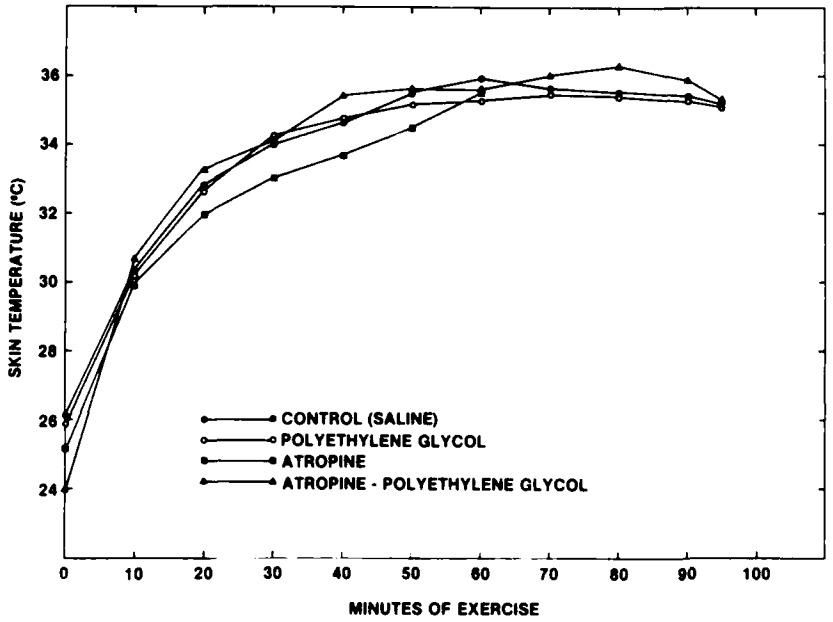

FIG. 2. Effects of polyethylene glycol, atropine, and a polyethylene glycol-atropine combination on skin temperature response to exercise in a warm environment. All conditions are as noted in Fig. 1.

TABLE 1. *Effects of PEG, ATR, and PEG-ATR on performance and thermoregulation during exercise in a warm environment*

	CONT	ATR	PEG	PEG-ATR
Endurance, min	67.9±5.1	50.3±3.7*	93.6±2.6†	76.3±4.8
Weight loss, g	12.2±0.8	6.3±0.4†	17.2±1.3†	10.4±1.3
Weight loss, g/min	0.184±0.01	0.123±0.01†	0.185±0.01	0.124±0.01†
ΔT_{re}, °C/min	0.043±0.004	0.067±0.005†	0.028±0.002*	0.041±0.004

Values are means ± SE; n = 10 in each group except ATR (n = 9). CONT, control; ATR, atropine; PEG, polyethylene glycol; ΔT_{re}, rectal temperature change. Significantly different from control: * $P < 0.05$; † $P < 0.01$.

TABLE 2. *Effects of PEG, ATR, and PEG-ATR on indexes of hypohydration before and subsequent to exercise in a warm environment*

	CONT		ATR		PEG		PEG-ATR	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Hematocrit, %RBC	41.1±0.3	39.05±0.3	42.5±0.6	38.2±0.7	36.2±0.6†	35.8±0.5†	36.6±0.6†	34.5±0.7†
Sodium, meq/l	140.6±0.3	144.3±0.5	140.7±0.5	143.9±0.6	137.7±0.4†	144.7±0.5	139.3±0.5	141.9±0.8
Osmolality, mosM/kg	298.2±0.8	308.8±1.0	297.4±0.9	303±1.1†	300.6±0.7	311.3±1.5	300.3±0.9	304.4±0.7†
Total protein, g/100 ml	6.6±0.08	6.2±0.09	6.6±0.06	5.8±0.08†	5.4±0.05†	5.4±0.06†	5.5±0.07†	5.3±0.06†

Values are means ± SE; n = 10 in each group except ATR (n = 9). Pre, preexercise; Post, postexercise; RBC, erythrocytes. See Table 1 footnote for definition of other abbreviations. Significantly different from respective control: * $P < 0.05$; † $P < 0.01$.

reduction in the PEG-ATR group cannot be explained on this basis. Total protein levels of both PEG-treated groups reflected the hemodilutional effects of this treatment before and subsequent to exercise in the warm environment. It is speculative that the postexercise reduction in total protein in the ATR-treated group may be related to the lowered osmolality of the respective sample and a physiological effect of the ATR.

Table 3 summarizes the effects of the treatments on several indexes of exercise duration and intensity. The results generally indicate that the exercise interval was sufficient to induce increments ($P < 0.05$) in lactic acid levels. Considerable variability in the responses of the enzymes creatine phosphokinase and lactate dehydrogenase precluded significant differences among the CONT, ATR, and PEG-ATR groups; however, postexercise levels of both enzymes in the longest running group (i.e., PEG) were significantly ($P < 0.01$, pre- vs. postexercise) increased by the exercise interval. Table 4 illustrates the effects of the treatments and exercise in a warm environment on several indexes of heat/exercise injury. Urea nitrogen and creatinine were consistently and significantly ($P < 0.01$) elevated by the exercise/heat regimen. Potassium levels were generally unaffected by the pharmacological treatments or the exercise regimen except in the PEG group wherein the exercise was accompanied by a reduction in concentration sufficient to elicit a significant ($P < 0.05$) difference from the respective control value.

DISCUSSION

As noted earlier, administration of hyperosmotic PEG solutions has been used extensively to effect acute dec-

TABLE 3. *Effects of PEG, ATR, and PEG-ATR on circulating indexes of exercise/heat stress before and subsequent to exercise in a warm environment*

	CONT		ATR		PEG		PEG-ATR	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Creatine phosphokinase, IU/l	62.6±3.6	200.9±49.4	48.8±3.2	137.3±14.1	36.1±1.8	453.8±182.8	34.0±2.2	109.5±17.0
Lactic acid dehydrogenase, IU/l	57.6±8.1	178±24.1	62±12	199.1±20.1	45.1±7.8	363.6±93†	31.3±3.3	136.7±24
Lactic acid, mg/100 ml	15.3±0.9	36.1±5.6	15.7±2.1	32.3±4.3	16.2±1.7	30.4±4.5	32.4±2.5*	47.9±3.9

Values are means ± SE; $n = 10$ for each group except ATR ($n = 9$). See Table 1 and 2 footnotes for definition of abbreviations. Significantly different from respective control: * $P < 0.05$; † $P < 0.01$.

TABLE 4. *Effects of PEG, ATR, and PEG-ATR on circulating indexes of exercise/heat stress before and subsequent to exercise in a warm environment*

	CONT		ATR		PEG		PEG-ATR	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Potassium, meq/l	4.7±0.1	4.8±0.3	5.4±0.2	5.3±0.2	4.7±0.1	4.0±0.2*	4.9±0.1	4.3±0.2
Urea nitrogen, mg/100 ml	19.6±1.0	26.9±1.3	18.0±0.6	25.8±1.2	17.3±0.6	25.7±1.4	20.5±1.3	24±1.1
Creatinine, mg/100 ml	0.5±0.03	0.8±0.1	0.5±0.03	0.8±0.06	0.5±0.04	0.7±0.02	0.5±0.03	0.7±0.04

Values are means ± SE; $n = 10$ in each group except ATR ($n = 9$). See Table 1 and 2 footnotes for definition of abbreviations. * Significantly different from respective control, $P < 0.05$.

rements in plasma volume in rats (25–27). Similarly, Horowitz and Nadel (14) administered 20% PEG to elicit 25% reductions in plasma volume in mongrel dogs while total body water was minimally affected. Generally, these and other reports have concluded that such reductions in plasma volume were accompanied by decrements in heat tolerance (14), acutely increased hematocrit (1, 23, 29), and similarly elevated circulating Na^+ concentrations (1, 24). Despite these physiological responses indicative of acute (6–12 h) intravascular hypohydration and therefore reduced capacity for exercise in heat, there was also evidence that the sequel to the initial decrement in plasma volume was a significant shift in fluid volume occurring from 24 to 72 h subsequent to PEG administration leading to expansion of plasma volume. Thus Stricker and MacArthur (29) reported a mean decrement in hematocrit of 4.2% 24 h after IP administration of PEG, and Denton (1) noted hematocrits falling from 43 to 38 and 32%, 48 and 72 h, respectively, after PEG injection into rabbits. To execute the current experiments, we had targeted mean plasma volume elevations of ~20%, and our preliminary experiments and calculations indicated that 24 h after an intraperitoneal injection of 3 ml of 30% PEG, such an increment was established.

This level of intravascular volume expansion provided marked physiological benefit to the PEG-treated rats in terms of increased endurance and decreased heating rate despite significantly prolonged and therefore increased salivary water loss during the treadmill interval. Although we had previously demonstrated that increasing hematocrit ratios to 52% (4) or infusing 2 ml NaCl or sodium bicarbonate (3) had no significant effects on subsequent exercise endurance in the heat, we had not previously manipulated plasma volume markedly in this rat model of human heat/exercise injury. However, preinduced hypothermia (7, 8) was shown to be very effective in increasing exercise endurance in a hot environment.

The current experiments indicated that the expanded plasma volume of PEG treatment provided some thermoregulatory benefit reflected in the reduced rate of heat gain secondary to the large water loss of the PEG-treated group. Although rats ordinarily require grooming behavior to optimize thermoregulatory benefit from salivation and usually receive modest benefit from salivation while running at 26°C, it is probable that in the current experiments at 30°C the large volumes of saliva lost during treadmill exercise provided a measure of passive spreading and evaporative cooling from the neck and mouth region and the ventral surface of the body. Possibly of equal importance, however, was the improved cardiovascular stability provided by the expanded plasma volume. We had previously reported (5) that in euhydrated rats increased cholinergic salivation during exercise in the heat did not elicit increased endurance. However, in the earlier experiments no manipulation of plasma volume had been attempted (i.e., rats were euhydrated), whereas in the current experiments plasma volume was significantly increased (i.e., hyperhydrated).

As noted earlier, we had previously demonstrated the efficacy of atropine and other anticholinergics in reducing both salivation and grooming behavior in passively heat-stressed rats (17, 21). However, it was uncertain whether the inhibition of salivation during exercise would be beneficial or detrimental to the physical performance and thermoregulation of the running rat. Benefit might be derived by preventing a reduction in the plasma volume loss that accompanies salivation, especially if this salivary water loss could not be fully translated to evaporative heat loss because of the inability to spread saliva behaviorally. Alternatively, a detriment could arise from a severe reduction in salivary water loss, which at 30°C prevented or attenuated any evaporative cooling effect passively derived from dripping saliva wetting the neck region and ventral surface of the body. The results of the current study indicate that the reduction in salivary water loss in the PEG-treated rats decreased

the thermoregulatory benefit and contributed to the reduced endurance capacity.

Compared with saline-treated controls, ATR rats displayed a mean decrement in endurance of 17.6 min; ATR rats that had been previously administered PEG manifested a 17.3-min reduction in endurance when compared with rats treated with PEG alone. While actively exercising on the treadmill, ATR-treated rats lost 0.061 g/min less body wt than saline-treated controls; ATR-PEG-treated rats also lost 0.061 g/min less than those treated with PEG only. Thus these results indicated that ATR alone reduces both endurance and salivary water loss when compared with saline treatment. When PEG was administered, total body water was increased by stimulated drinking and fluid retention; this was followed by plasma volume expansion as evidenced in significantly reduced hematocrit and hemoglobin levels. After plasma volume expansion, endurance was prolonged and water loss was elevated in a time-dependent fashion compared with saline-treated controls. ATR administration to rats previously treated with PEG reduced performance and weight loss to approximately control levels. Thus ATR had consistent detrimental effects on saline-treated rats and reduced the advantages of PEG administration to approximately control levels. The adverse effects of ATR appear to be thermoregulatory in nature and probably also dependent on the ambient temperature at which the exercise is carried out.

The hemodynamic data noted in Table 2 are particularly worthy of comment and interpretation. These data indicate that both hematocrit and total protein have decreased during the exercise interval, whereas osmolality and plasma sodium levels have generally increased. These apparent dichotomies are worthy of future investigation, but presently, several factors may be relevant in providing a plausible explanation. Initially it should be noted that the consecutive removal of two blood samples, despite the small volumes taken (0.8 ml), could account for a 2% decrement in hematocrit in the pre- to postexercise blood samples. Additionally Van Beaumont et al. (30) reported that mean erythrocyte volume in humans can be decreased during exercise in a warm environment if plasma osmolality is increased by >5 mosM/kg, a condition present in the current experiments. Both these mechanisms then could have contributed to a decreased hematocrit in the postexercise blood samples independent of plasma Na^+ levels or osmolality. Furthermore, if interstitial fluid entered the intravascular volume during the exercise interval, the sodium concentration of the plasma could have been increased slightly, whereas its protein concentration was decreased; Pitts (23) reported that the Na^+ concentration of the interstitial fluid is slightly greater than that of plasma, whereas the protein concentration of interstitial fluid is negligible. Of course the elevated osmolality in the postexercise plasma can be reasonably explained by increments in postexercise Na^+, urea nitrogen, creatinine, lactate, and other metabolites.

Clinical indexes of heat/exercise injury generally mirrored the dilutional effects of the PEG administration and the longer run time elicited by this treatment. The reduced plasma osmolality and circulating protein in the postexercise samples of the ATR group (compared with CONT) may reflect the decreased endurance of this group with attendant insufficient equilibrium time for protein to be returned to the circulatory system during the exercise interval. However, direct effects of ATR cannot be ruled out. The significant reduction in plasma potassium levels in the PEG-treated rats has been observed previously (6) in rats exercising lightly with the achievement of steady-state T_{re}. When exercise is accompanied by marked hyperthermia, we ordinarily observed significant elevations of circulating potassium levels, although these earlier experiments (5, 9–11) were conducted at 35°C ambient temperature.

We concluded from these experiments that plasma volume expansion secondary to PEG administration improved exercise performance and thermoregulation. During exercise, total water loss among PEG-treated animals was significantly greater than that of saline-treated controls. ATR reduced physical performance and salivary water loss in both saline- and PEG-treated rats. Hydrational markers and clinical chemical indexes of heat/exercise injury suggested that the beneficial effects of PEG and detrimental effects of ATR were attributable primarily to increased intravascular volume and decreased salivation, respectively.

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Address for reprint requests: R. P. Francesconi, Heat Research Division, US Army Research Institute of Environmental Medicine, Natick, MA 01760-5007.

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